

CLAIMS

*Sub A2*

1. Method of amplifying any RNA target sequence, by transcription under the control of a promoter, in an RNA sample comprising said target sequence, in which said sample is brought into contact:
  - with a reagent capable of hybridizing with said RNA comprising said target sequence,
  - and with an enzymatic system comprising an RNA-dependent RNA polymerase activity, under conditions allowing the hybridization of said reagent with said RNA comprising said target sequence and under conditions allowing the functioning of said RNA-dependent RNA polymerase activity;
- 10 in which said reagent contains:
  - (i) a first nucleotide strand comprising: a) a first nucleotide segment capable of playing the role of sense strand of a promoter for said RNA polymerase activity and b), downstream of said first segment, a second nucleotide segment comprising a sequence capable of hybridizing with a region of said RNA, and
  - 20 (ii), in the hybridized state on the first strand, a second nucleotide strand comprising a third nucleotide segment capable of hybridizing with said first segment so as to form with it a functional double-stranded promoter;
- 25 and in which said RNA polymerase activity is capable of transcribing an RNA template, in the presence of said reagent hybridized with said template, in the absence of associated protein factor and in the absence of a ligase activity.
- 30 2. Method according to claim 1, in which said third segment is flanked, at its upstream end, by a fourth nucleotide segment which is shorter than said second segment of the first strand.

3. Method according to claim 2, in which said fourth segment is capable of hybridizing with a portion opposite said second segment.

4. Method according to either of claims 2 and 3, in which said fourth segment of said second strand is chosen from those whose sequence facilitates the initiation of transcription for said RNA polymerase.

5. Method according to any one of claims 2 to 4,

10 5. Method according to any one of claims 2 to 4, in which said second segment of said first strand contains a number of nucleotides at least equal to the sum of the number of nucleotides of said fourth segment, if it is present, and of the number of nucleotides of said sequence of the second segment which is capable of hybridizing with said region of said RNA.

15 6. Method according to any one of the preceding claims, in which said first and third segments consist of DNA.

7. Method according to any one of the preceding 20 claims, in which said fourth segment consists of DNA.

8. Method according to any one of the preceding claims, in which said RNA polymerase is a virus or phage wild-type RNA polymerase.

9. Method according to claim 8, in which said RNA 25 polymerase is chosen from the family of RNA polymerases including the T7 RNA polymerase, T3 RNA polymerase and SP6 RNA polymerase.

10. Method according to claim 8, in which said RNA polymerase is derived by mutation from an RNA polymerase chosen from the family of RNA polymerases 30 including the T7, T3 and SP6 RNA polymerases.

11. Method according to claim 10, in which said RNA polymerase contains at least one mutation in the region corresponding to the T7 RNA polymerase sequence 35 containing amino acids 625 to 652.

12. Method according to claim 11, in which said RNA polymerase is capable of transcribing a polynucleotide

target sequence with a better yield when said target sequence consists of RNA than when it consists of DNA.

13. Method according to any one of the preceding claims, in which said enzyme system contains only an RNA polymerase activity.

5 14. RNA polymerase which can be used in the method of any one of the preceding claims, capable of transcribing, under the control of a promoter, a polynucleotide target of interest of any sequence contained in a polynucleotide template, by synthesizing, in the presence of said template and in the absence of associated protein factor, a product of transcription containing an RNA sequence complementary to said sequence, said RNA polymerase being capable of synthesizing said product of transcription with a better yield when said target sequence of said template consists of RNA than when it consists of DNA.

10 15. RNA polymerase according to the preceding claim, in which the ratio of the yield of product of transcription of the RNA template to the yield of product of transcription of the DNA template is greater than 2 and in particular greater than 10.

16. RNA polymerase according to either of claims 14 and 15, characterized in that it is derived by mutation from a virus or phage RNA polymerase.

20 17. RNA polymerase according to claim 16, characterized in that said phage is an E.coli phage.

25 18. RNA polymerase according to any one of claims 14 to 17, characterized in that it possesses a protein sequence homology greater than 50%, and in particular greater than 80% with a wild-type RNA polymerase of the family of DNA-dependent RNA polymerases including the T7 RNA polymerase, T3 RNA polymerase and SP6 RNA polymerase.

30 35 19. RNA polymerase according to claim 18, characterized in that it contains at least one mutation in a region corresponding to the T7 RNA polymerase sequence containing amino acids 625-652.

*a2*

20. RNA polymerase according to claim 19, characterized in that it has the composition of a wild-type DNA-dependent RNA polymerase, with the exception of the fact that it contains at least one mutation in said region.

21. RNA polymerase according to claim 19 or 20, characterized in that it contains at least one mutation at a position corresponding to one of positions 627, 628, 631, 632 and 639 of the T7 RNA polymerase amino acid sequence.

22. RNA polymerase according to any one of claims 19 to 21, characterized in that said mutation comprises the replacement of an amino acid residue, chosen from arginine, lysine, serine and tyrosine, of the wild-type RNA polymerase, with another amino acid residue.

23. RNA polymerase according to claim 22, characterized in that said amino acid replaced is an arginine or a lysine and/or in that said other amino acid residue is an alanine, valine, leucine, isoleucine, glycine, threonine or serine residue.

24. RNA polymerase according to any one of claims 19 to 23, characterized in that said mutation comprises the replacement of all or part of said region with a homologous region present in a wild-type RNA-dependent polymerase.

25. Gene encoding an RNA polymerase as defined in any one of claims 14 to 24.

26. Expression vector into which a gene as defined in the preceding claim is inserted, said vector being capable of expressing said RNA polymerase in a host cell.

27. Host cell containing an expression vector as defined in the preceding claim.

28. Method of producing an RNA polymerase as defined in any one of claims 14 to 24, characterized in that: a) a gene encoding a wild-type RNA polymerase is obtained in a known manner, b) at least one mutation is performed on said gene, c) the mutated gene obtained is

5

properties of an RNA polymerase as defined in either of claims 14 and 15 are selected.

29. Use of an RNA polymerase capable of transcribing an RNA template, under the control of a promoter, in the absence of auxiliary protein factor, in a method of transcription of a template strand comprising an RNA target sequence, in which said RNA polymerase is chosen from the T7 RNA polymerase, the SP6 RNA polymerase and the RNA polymerases as defined in any one of claims 14 to 24.

30. Use of an RNA polymerase capable of transcribing an RNA template, under the control of a promoter, in the absence of auxiliary protein factor, in a method of transcription of a template strand comprising an RNA target sequence, in which said template strand consists of RNA from one of positions +1 to +5 up to the 5' end of the template strand, and consists of DNA from said position up to the 3' end of the template strand when said 3' end does not coincide with said position.

31. Use according to the preceding claim, in which said RNA polymerase is a virus or phage wild-type RNA polymerase.

30 32. Use according to the preceding claim, in which said RNA polymerase is chosen from T7, T3 and SP6 RNA polymerase.

Add  
a3